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PRINCIPAL INVESTIGATOR: Yong Zhuang, Ph.D.

CONTRACTING ORGANIZATION: Weill Medical College of Cornell University  
New York, New York 10021

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<b>13. ABSTRACT (Maximum 200 Words)</b> I have previously identified some RAR $\beta$ targets genes. In the past year, I carried out actinomycin D experiments and determined that the RAR $\beta$ targets genes are transcriptionally regulated by RAR $\beta_2$ . In addition, I have shown that RAR $\beta$ selective agonists and RXR agonists synergistically induce RAR $\beta_2$ target genes in F9 wild-type cells. I also showed that ELF3, one of RAR $\beta$ target genes, was up-regulated by RA in normal human prostate epithelial cells, but not in human prostate tumor cells.				
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## Introduction

Retinoids, a group of natural and synthetic analogues of vitamin A (retinol), can modulate cell growth and differentiation *in vitro* and *in vivo* (Gudas et al., 1994). An inverse relationship between retinoids levels and carcinogenesis has been observed over the last two decades. Vitamin A deficiency in experimental animals is associated with a higher incidence of cancer and with increased susceptibility to chemical carcinogens (Moon et al., 1994). Further, epidemiological studies showed that individuals with a lower dietary vitamin A intake are at a higher risk to develop cancer (Hong and Itri, 1994). Experimental models of carcinogenesis have demonstrated the efficacy of pharmacological levels of retinoids in preventing the development of cancers of the skin, oral cavity, lung, mammary gland, prostate, bladder, liver, and pancreas in animals exposed to carcinogenic agents (Moon et al., 1994). Thus, strong evidence exists that retinoids can prevent some types of cancers. Much *in vitro* and *in vivo* data indicate that retinoids play a role in the prevention of prostate cancer (Nanus and Gudas, 2000).

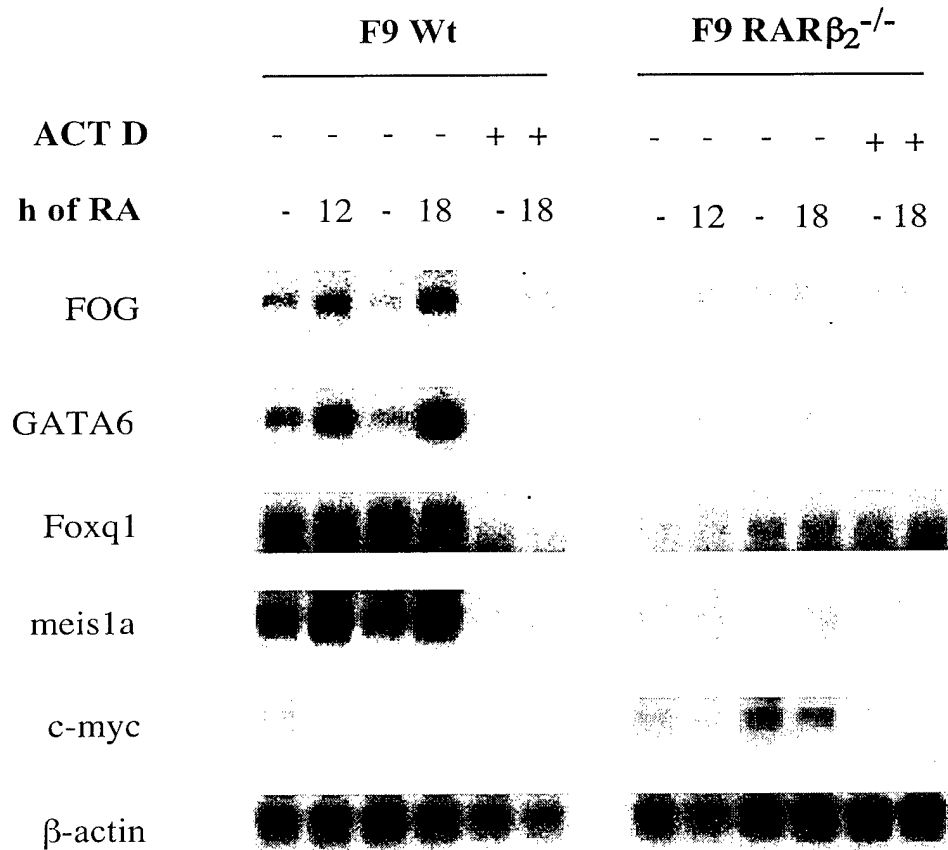
The biological effects of retinoids are mainly mediated by two classes of nuclear retinoid receptors: retinoic acid receptors (RARs) and retinoid X receptors (RXRs) (Mangelsdorf et al., 1994). The receptor RAR $\beta$  is not expressed in a number of malignant tumors, including prostate cancer, lung carcinoma, squamous cell carcinoma of the head and neck, breast cancer, and esophageal carcinoma (Hu et al., 1991; Swisshelm et al., 1994; Zhang et al., 1994; Xu et al., 1994; Qiu et al., 1999). It has been suggested that the decrease in RAR $\beta$  expression may lead to resistance to the growth inhibitory actions of retinoids. Indeed, transfection of RAR $\beta$  into RAR $\beta$ -negative cervical, breast, and lung cancer cells increased cell responsiveness to growth inhibition and induction of apoptosis by retinoids (Li et al., 1998; Si et al., 1996; Seewaldt et al., 1995; Liu et al., 1996; Weber et al., 1999). Stable expression of RAR $\beta$  in a RAR $\beta$ -negative prostate cancer cell line, PC-3, also potentiates the growth inhibitory effects of vitamin D3 analog and retinoids (Campbell et al., 1998).

In order to understand the function of the transcription factor RAR $\beta$ , it is imperative that target genes regulated by RAR $\beta$  are identified and analyzed. I have already successfully performed experiments to identify some of the target genes of RAR $\beta$  in F9 cells by the use of subtractive hybridization and DNA expression micorarray techniques. By employing these two methods, I have identified many target genes, including transcription factors, protein tyrosine kinases, homeobox proteins, oncoproteins, ion channels, etc. Northern blot analysis was used to examine the regulation of the expression of these putative RAR $\beta$  target genes by RA both in F9 Wt and F9 RAR $\beta_2$ <sup>-/-</sup> cells.

## Body

I carried out actinomycin D experiments to determine if the target genes I identified are transcriptionally regulated by RAR $\beta$ . F9 wild type cells and F9 RAR $\beta_2$ <sup>-/-</sup> cells were first treated for 12 h with or without 1  $\mu$ M RA. At 12 h, RNA was isolated from some dishes of cells, while actinomycin D (2  $\mu$ g/ml) was added to other dishes of cells to inhibit RNA transcription. RNA was isolated after an additional 6 h. The RNA samples were analyzed by Northern blot analysis (Figure 1). RA induced the target gene expression in F9 wild-type cells, but not in the F9 RAR $\beta_2$ <sup>-/-</sup> cells. Treatment of wild type

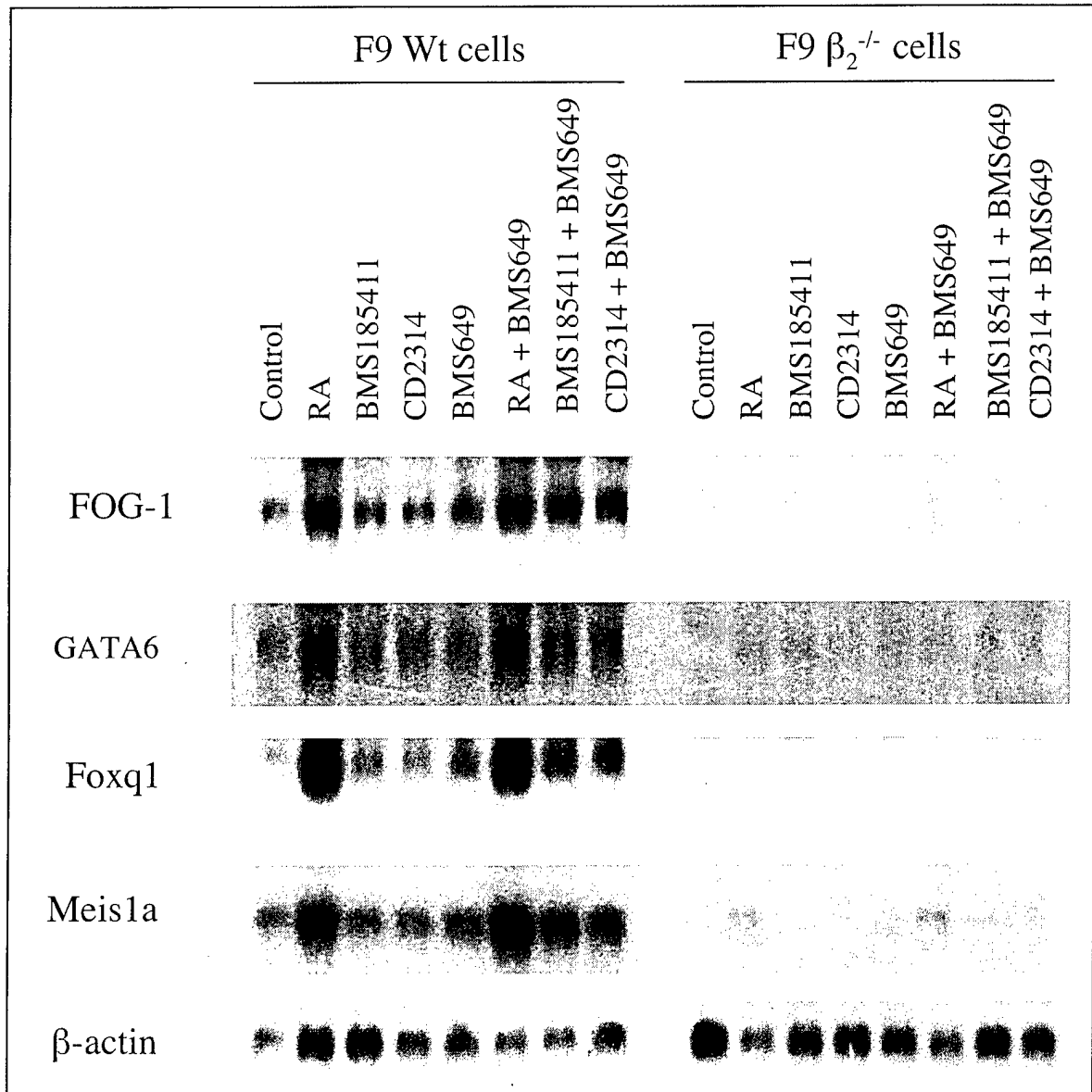
cells with actinomycin D alone resulted in a decrease in target gene mRNA. Furthermore, RA induction of target gene mRNA was completely blocked by treatment with actinomycin D, indicating that ongoing transcription is required for RA-induced expression of target genes. For c-myc, the mRNA level seen after 18 h of RA treatment in F9 RAR $\beta_2^{-/-}$  cells was greatly reduced after 6 h of actinomycin D treatment, which is consistent with the previous finding that the half-life of c-myc transcripts in F9 cells is about 40 min.



**Figure 1.** Northern blot analysis of the effects of actinomycin D on RAR $\beta_2$  target gene expression. Wild-type F9 cells and F9 RAR $\beta_2^{-/-}$  cells were grown for 12 h in the absence (-) or presence (+) of 1  $\mu$ M RA. At 12 h, RNA was isolated or 2  $\mu$ g/ml transcriptional inhibitor actinomycin D (ACT D) was added to the medium and RNA was isolated after 6 h. After size-fractionation on the gel, the RNA was transferred onto nylon and hybridization to different cDNAs for the target genes was performed. This experiment was performed twice with similar results; one experiment is shown.

I also carried out agonist assays. F9 Wt and RAR $\beta_2^{-/-}$  cells were treated for 48 h with 1  $\mu$ M RA or RAR $\beta$  selective agonists (100 nM BMS185411 and 1  $\mu$ M CD2314), and/or 1  $\mu$ M pan-RXR agonist (BMS188,649 [BMS649]). BMS185411 or CD2314 alone didn't induce the expression of any target genes in F9 Wt cells. Similarly, BMS649 (pan-RXR) alone had no effect on the expression of target genes. However, BMS649 (pan-

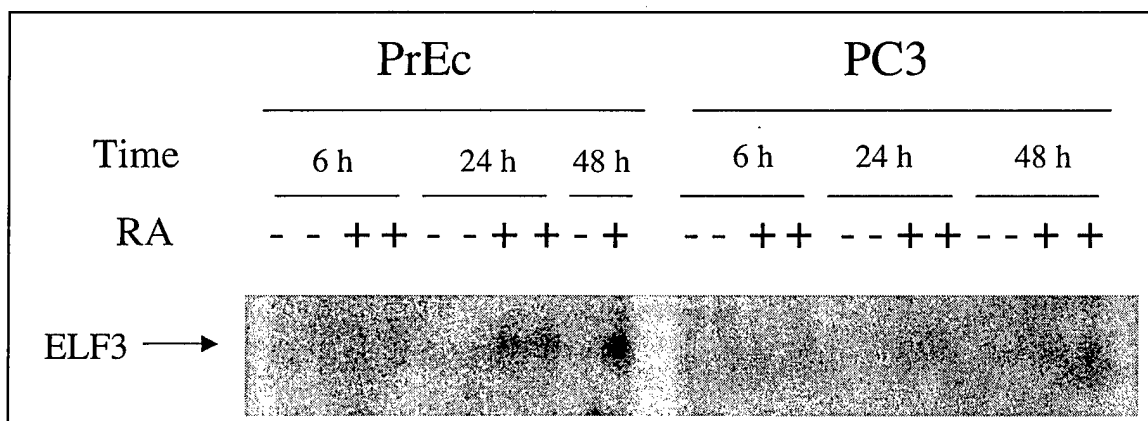
RXR) and the RAR $\beta$  selective agonists (BMS185411 or CD2314) synergistically induced the expression of all target genes in F9 Wt cells, but not in F9 RAR $\beta_2^{-/-}$  cells (Figure 2). Thus, RXR-RAR $\beta_2$  heterodimers appear to play a major role in mediating the induction of the expression of RAR $\beta_2$  target genes.



**Figure 2.** Expression of RAR $\beta_2$  target genes is increased by a RAR $\beta$ -specific agonist and pan-RXR agonist. F9 wild-type cells and RAR $\beta_2^{-/-}$  cells were treated for 48 h with 1  $\mu$ M RA, 1  $\mu$ M or 100 nM RAR $\beta$ -specific agonist (BMS185411 or CD2314), 1  $\mu$ M pan-RXR agonist (BMS188649), or different combinations of drugs. Total RNA was then extracted and size-fractionated on the gel. The RNA was transferred onto nylon and hybridized to different cDNAs for the target genes. The experiments have been repeated two times with different RNA preparations.  $\beta$ -actin is used as an RNA loading control. One representative experiment is shown here.

ELF3 is a new RAR $\beta$  target I have identified. ELF3 is a novel member of the Ets family of transcription factors. ELF3 is found to be specifically expressed in epithelial cells and in adult human tissues such as prostate, lung and kidney, which contain a significant proportion of epithelial cells (Tymns et al., 1997). ELF3 plays a role in epithelial differentiation and has been shown to suppress basal keratin 4 promoter activity in both esophageal and cervical epithelial cancer cell lines (Brembeck et al., 2000). Thus, ELF3 may play an important role in epithelial cancers of the prostate. I have also shown that the ELF3 mRNA level is lower in the lung and kidneys from RAR $\beta$  knockout mice than from wild type mice.

I thus tested the expression of ELF3 mRNA in normal human prostate epithelial cell strains vs. prostate cancer cell lines. I carried out this study by using PrEc cells, a normal prostate epithelial cell line, and PC3 cells, a prostate cancer line. These cells were treated with 1 $\mu$ M RA for different times, mRNA was extracted, and Northern analysis was performed. ELF3 mRNA is not detectable in either control PrEc cells or PC3 cells. RA treatment for 24 h and 48 h resulted in a 5-fold induction of ELF3 mRNA in PrEc cells, but did not have an effect in PC3 cells (Figure 3). Since PC3 cells do not express RAR $\beta$ , this result suggests that key RAR $\beta$  target genes are not properly regulated in human prostate cancer as compared to normal cultured human prostate epithelial cells. I am currently examining expression of many other RAR $\beta$  target genes in PrEc and PC3 cells.



**Figure 3.** PrEC cells and PC3 cells were treated for 6 h, 24 h and 48 h with 1  $\mu$ M RA, Total RNA was then extracted and size-fractionated on the gel. The RNA was transferred onto nylon and hybridized to cDNA for ELF3.

## Key research Accomplishments

- I carried out Actinomycin D experiments to determine if the target genes are transcriptionally regulated by RAR $\beta_2$ .
- I determined whether RAR $\beta$  agonists and /or RXR agonists can stimulate the expression of the RAR $\beta$  target genes.
- I studied the effects of retinoic acid on the expression of key RAR $\beta$  target genes (eg. ELF3) in normal human prostate epithelial cells and prostate tumor cells.

## Conclusions

I conclude that the RAR $\beta$  targets genes are transcriptionally regulated by RAR $\beta_2$ . In addition, I have shown that RAR $\beta$  selective agonists and RXR agonists synergistically induce RAR $\beta_2$  target genes in F9 wild-type cells. I also showed that ELF3, one of RAR $\beta$  target gene, was up-regulated by RA in normal human prostate epithelial cells, but not in human prostate tumor cells.

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